ADENOSINE TRIPHOSPHATE FROM INSECTS

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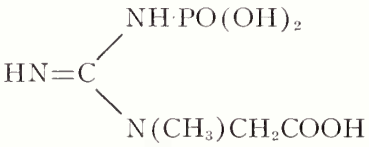
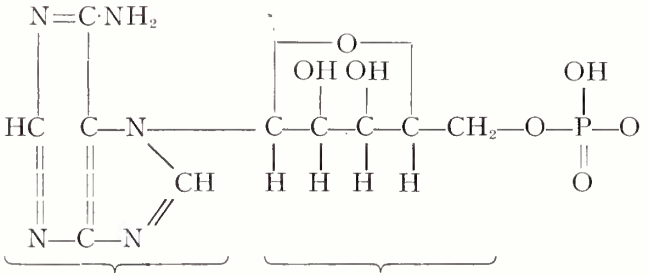
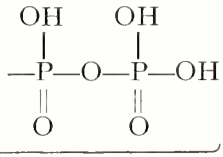
In recent years the attention of the biochemist has become focussed on the way in which light energy from the sun, converted by the green plant into carbohydrate, is made available for vital function in living tissue. It is now generally established that this carbohydrate (usually in the form of glucose), does not make its energy directly available for cell function, but rather transfers its energy in small parcels, through the activity of enzymes, to specialized molecules which act as the immediate energy donors for reactions of all kinds.

Of several such compounds that are known, two are of special interest: phosphocreatine and adenosine triphosphate (ATP); the formulae of these are shown below:



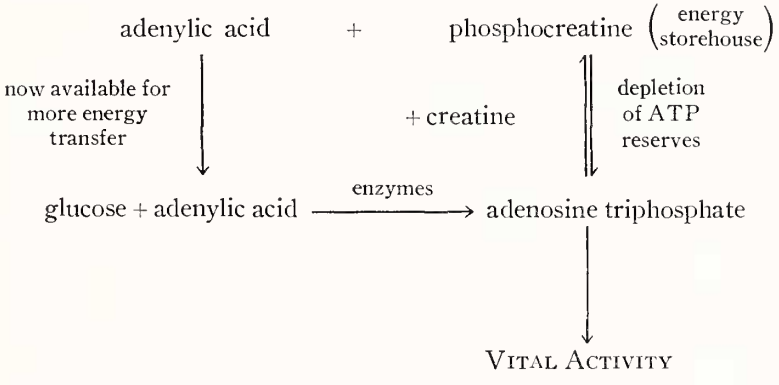
phosphocreatine





adenosine triphosphate

The energy originally resident in the glucose molecule appears in these compounds in the N—P bond of phosphocreatine and in the P—O—P bonds of adenosine triphosphate (high energy phosphate bonds). Adenosine triphosphate is the immediate recipient of the glucose energy. Therefore, one would expect that in the organism the quantity of adenosine triphosphate which could be formed would be limited by the quantity of adenylic acid (see above formula) which can act as an energy acceptor. The organism, however, has another mechanism for increasing its energy stores. As soon as the adenylic acid has been "saturated" with high energy phosphate bonds, these are temporarily transferred to creatine to form phosphocreatine; more energy can now be taken up by the "adenylic acid system." When energy must be used for vital activity, adenosine triphosphate acts as the immediate donor. As soon as its level falls, it is replenished from the phosphocreatine reservoir. These reactions are shown in a general way in the following equation:



Phosphocreatine and adenosine triphosphate are found in all vertebrates. In invertebrates, phosphocreatine is replaced by a related compound, phosphoarginine. The kind of adenosine triphosphate which is present in invertebrates, however, has not yet been extensively investigated. The remainder of the present paper concerns itself with the adenosine triphosphate of insects.

We have succeeded in isolating adenosine triphosphate from adults of *Drosophila melanogaster* Meigin (Diptera). Since this compound is very unstable and breaks down rapidly on the death of the animal, the isolation must be carried out on freshly killed animals and all manipulations must be carried out in the cold.

The procedure employed in the isolation was essentially that of Needham (1) with slight modification. A typical run is outlined below: 11.5 g. of ether-anesthetized *Drosophila melanogaster* were homogenized in approximately 10 ml. of iced 10% trichloracetic acid with a motor driven glass homogenizer, and the protein removed by centrifugation. To the orange-pigmented supernatant fluid was added an equal volume of cold 95% ethyl alcohol; the precipitated glycogen was centrifuged out. To the supernatant were added 3 ml. of 25% barium acetate and the pH adjusted to 7.0 with 30% NaOH. After 0.5 hour in the cold the barium precipitate was collected and washed twice with cold 95% alcohol. (This removed most of the orange pigment.) The barium precipitate was then suspended in water, centrifuged once more and the supernatant discarded. The barium-insoluble precipitate remaining was then treated according to the method of Needham (1) for the isolation of ATP from mammalian muscle, and 20 mg. of a grayish barium salt were obtained.

The barium salt was assayed for inorganic phosphorus, labile phosphorus (phosphorus hydrolyzed in 7 minutes at 100° C. in N HCl) and total phosphorus, according to the method of Fiske and SubbaRow (2). Color development curves for pentose were run according to the method of Albaum and Umbreit (3). Adenine was assayed spectrophotometrically.

On the assumption that the compound isolated was barium adenosine triphosphate (M.W. 853), the purity based on organic phosphorus was 78%. The molar ratio of labile phosphorus: total phosphorus: pentose: adenine was 1.90: 3.00: 1.00: 1.04.

The adenosine triphosphate isolated from Drosophila appears to be identical with that obtained from vertebrate muscle. This identity has been established on the basis of the molar concentration of adenine: ribose: phosphorus, as well as on the basis of physiological activity.

As indicated above, adenosine triphosphate is isolated from the barium insoluble precipitate. If such a precipitate is prepared from other organisms, where not much material is available, it is possible by simply analyzing this precipitate to qualitatively demonstrate the presence of this compound (phosphorus, adenine, and ribose content). This kind of experiment has been done on the following insects:

COLEOPTERA

Tribolium confusum Jacq. Duz., adults.

Tenebrio molitor L., larvae.

ISOPTERA

Reticulitermes flavipes Kol., workers

ORTHOPTERA

Paratenodera sinensis Sauss., nymphs

Adenosine triphosphate appears to be present in all of these, and

on the basis of the analytical data resembles that isolated from *Drosophila melanogaster*.

SUMMARY

A procedure is described for isolating adenosine triphosphate from insects. This compound has been isolated from *Drosophila melanogaster* and its presence demonstrated in four other insects. On the basis of analytical and physiological data, the isolated compound is identical with that obtained from vertebrate tissue.

LITERATURE CITED

1. **Needham, D. M.** 1942. Biochem. J. 38, 121.
2. **Fiske, C. H. & SubbaRow, Y.** 1924. J. Biol. Chem. 66, 375.
3. **Albaum, H. G. & Umbreit, W. W.** 1947. J. Biol. Chem. 167, 369.